Detection of CYP2C12 in Formalin-Fixed, Paraffin-Embedded Rat Tissue

Reagents:
1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
Citrate Buffer
DAB Chromagen
Hematoxylin

Antibody Information:
Blocking serum: Normal Goat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 005-000-121

Avidin Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #SP-2001

Primary antibody: Rabbit Polyclonal to Cytochrome P450 2C + 2C9 + 2C19 + 2C12
Abcam
Cambridge, MA 02139
www.abcam.com
1-888-772-2226
Catalog# ab22596-50

Negative control serum: Normal Rabbit Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #011-000-001

Secondary antibody: Biotinylated goat anti-rabbit IgG
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # BA-1000

Label antibody: Peroxidase –conjugated Streptavidin SS Label
Biogenex
San Ramon, CA 94583
www.biogenex.com
1-800-421-4149
Catalog # HK330-9K

Staining Procedure

Positive Control Tissue: Rat Liver (upregulated)
Stain Localization: Centrilobular cytoplasmic staining pattern

Deparaffinize and hydrate slides through the following solutions:

Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.

2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

3. Unmasking Technique using the decloaker.
   Add 500ml distilled water to the pan of the decloaker.
   Place a full rack of slides in a Tissue Tek™ container containing 250ml of 1X citrate buffer solution.
   Decloak for 5 minutes. Pressure_____
   Depressurize for 10 minutes.
   Remove pan top and cool for 10 minutes. Temperature after cooling _____
   Rinse in distilled water two times for 3 minutes each.

4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

5. Block with 10% Normal Goat Serum for 20 minutes at room temperature.
Lot#_____ Reconstituted Date_____

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN.

6. Apply Avidin/Biotin block
   Lot#_____ Exp. Date_____New Kit: yes / no
   Apply avidin block - 15 minutes at room temperature.
   Quick rinse in 1X Automation Buffer
   Apply biotin block - 15 minutes at room temperature.
   Wipe excess block

   DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (Cyp2C12) at 1:1000 dilution and incubate for one hour at room temperature.
   Lot#_____ Date Aliquoted_____

   For negative control slides, normalize the normal rabbit serum to match the protein concentration of the primary antibody (Cyp2C12), and use this to make a 1:1000 dilution. Apply to the slides and incubate for one hour at room temperature.
   Lot#_____ Reconstituted Date _____

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

9. Apply Goat anti-rabbit secondary antibody at a 1:500 dilution and incubate for 30 minutes at room temperature.
   Lot#_____ Reconstituted Date_____

10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

11. Apply the Biogenex Supersensitive Label and incubate 30 minutes at room temperature.
    Lot#_____ Exp. Date_____

12. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

13. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
    (Add 1 drop of DAB per ml of substrate)
    Lot#_____ Exp. Date_____ New Kit: yes / no

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 20 seconds.

16. Rinse in tap water until water is clear.

17. Gently agitate slides in 1X Automation Buffer until blue.

18. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% Ethanol	3 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip

Updated 08/21/06